(FILE 'HOME' ENTERED AT 17:39:50 ON 15 OCT 2003)

FILE 'MEDLINE, EMBASE, CANCERLIT, BIOTECHDS, BIOSIS' ENTERED AT 17:40:17 ON 15 OCT 2003

L1	1200771	S	CALCIUM OR CA
L2	592501	S	LIPID OR LIPOSOME OR LIPOFECTIN OR DODAP OR DODAC OR DDAB OR
L3	32882	S	L2 AND L1
L4	6597724	S	INCREAS? OR ENHANC?
L5	15080	S	L4 AND L3
L6	2317771	S	ENDOC? OR ENDOSOME OR TRANSFE?
L7	1611	S	L6 AND L5
L8	2502306	S	DNA OR NUCLEIC OR PLASMID
L9	316	S	L8 AND L7
L10	201		DUP REM L9 (115 DUPLICATES REMOVED)
L11	19577	S	CALCIUM IONS
L12	2915	S	CA IONS
L13	22220	S	L12 OR L11
L14	4	S	L13 AND L10
L15	38	S	L10 AND CATIONIC

L14 ANSWER 2 OF 4 EMBASE COPYRIGHT 2003 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
AN 1998021514 EMBASE
TI Mechanism of **calcium** ion induced multilamellar vesicle-**DNA** interaction.
AU Mozafari N.R.; Hasirci V.
CS V. Hasirci, Middle East Technical University, Biotechnology Research Unit, Biotechnology Research Unit, Ankara 06531, Turkey
SO Journal of Microencapsulation, (1998) 15/1 (55-65).
Refs: 40
ISSN: 0265-2048 CODEN: JOMIEF
CY United Kingdom
DT Journal; Article
FS 022 Human Genetics
027 Biophysics, Bioengineering and Medical Instrumentation
030 Pharmacology
037 Drug Literature Index
039 Pharmacy
LA English
SL English
AB The effect of Ca2+ on the **DNA** interaction with anionic and neutral multilamellar vesicles (MLV) has been investigated. **DNA** from wheat (Triticum aestivum L. Gerek) was introduced to a suspension of MLV, composed of phosphatidylcholine (PC):dicetylphosphate (DCP):cholesterol (CHOL) at different molar ratios, to which Ca2+ (5-75 mM) was subsequently added. Indication of aggregation and/or fusion was obtained via light-scattering examination following the addition of Ca2+ and **DNA** to the MLV medium. Using a UV spectrophotometric assay, it was observed that although **DNA** alone has no effect on negatively charged MLV, it **enhances** liposomal interaction in the presence of **calcium ions**. The minimal Ca2+ concentration required to promote the interaction was detected to be 10 mM, and the highest level of interaction was observed at 75 mM. The aggregation/fusion of vesicles was detected for uncharged MLV (with no DCP in their structure), as well as for the anionic ones containing c. 10% CHOL, but not for anionic MLV containing 40% CHOL. This is explained in terms of cholesterol decreasing the membrane fluidity (above the Tc of components) as a result of which more rigid vesicles become less prone to aggregation/fusion interactions.

aggregation/fusion interactions.

L14 ANSWER 3 OF 4 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
AN 1997-13284 BIOTECHDS
TI **Increasing** or decreasing **transfection** efficiency; new lipofection method and cationic **liposome** for gene **transfer**, and ribozyme and antisense oligonucleotide delivery in infection and cancer gene therapy
AU Mislick K A
PA California-Inst.Technol.
LO Pasadena, CA, USA.
PI WO 9734483 25 Sep 1997
AI WO 1997-US4217 12 Mar 1997
PRAI US 1996-644095 10 May 1996; US 1996-13647 18 Mar 1996
DT Patent
LA English
OS WPI: 1997-489242 [45]
AB A method for administering genetic material (preferably **DNA**, RNA, mRNA, ribozymes, antisense oligonucleotides, modified polynucleotides, modified oligonucleotides or combinations) to cells (preferably fibroblasts, myoblasts, hepatocytes, cells of hematopoietic origin (such as white blood cells and bone marrow cells), cancer cells, ischemic tissue, neurons and other cells of the nervous system, and non-differentiated cells) is claimed, which involves in vitro, in vivo or ex vivo administration of an effective amount of complex genetic material and a cationic species, and an effective amount of a compound that **increases** proteoglycan expression on the cell surface to **increase** the **transfection** efficiency relative to when the cells exhibit normal proteoglycan expression. The cationic species is selected from the group consisting of cationic lipids, cationic liposomes, **calcium ions**, lipopolyamine, polyethylene imine, polycationic amphiphiles, DEAE-dextran and dendrite polymers containing functional groups. Also claimed are a method for decreasing the efficiency of **transfection** and an improved **lipid**. The method can be used for infection or cancer gene therapy. (64pp)

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     (FILE 'MEDLINE, CANCERLIT, BIOTECHDS, EMBASE, BIOSIS' ENTERED AT 16:42:11
     ON 14 OCT 2003)
                DEL HIS
L1       1200654 S CA OR CALCIUM
L2        593059 S CATIONIC LIPID OR CATIONIC LIPOSOME OR LIPOFECTIN OR AMPHIPHI
L3         32968 S L1 AND L2
L4         28799 S ENDOSO?
L5            90 S L4 AND L3
L6            56 DUP REM L5 (34 DUPLICATES REMOVED)
L7           116 S L1 AND (CATIONIC LIPID OR CATIONIC LIPOSOME) AND (NUCLEIC OR
L8            66 DUP REM L7 (50 DUPLICATES REMOVED)

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L8 ANSWER 47 OF 66 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 1998:457658 BIOSIS
DN PREV199800457658
TI Incorporation of **calcium** into **cationic liposome-plasmid DNA** complexes significantly increases cell transfection in vitro.
AU Lam, Man Iu; Cullis, Pieter
CS Dep. Biochem. and Mol. Biol., Fac. Med., Univ. B.C., Vancouver, BC V6T 1Z3 Canada
SO Journal of Liposome Research, (Feb., 1998) Vol. 8, No. 1, pp. 75-76.
Meeting Info.: Sixth Liposome Research Days Conference Les Embiez, France May 28-31, 1998
ISSN: 0898-2104.
DT Conference
LA English

L8 ANSWER 36 OF 66 MEDLINE on STN DUPLICATE 11
AN 2000141066 MEDLINE
DN 20141066 PubMed ID: 10675506
TI **Calcium** enhances the transfection potency of **plasmid DNA-cationic liposome** complexes.
AU Lam A M; Cullis P R
CS Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of British Columbia, 2146 Health Sciences Mall, Vancouver, BC, Canada.. milam@interchange.ubc.ca
SO BIOCHIMICA ET BIOPHYSICA ACTA, (2000 Feb 15) 1463 (2) 279-90. Journal code: 0217513. ISSN: 0006-3002.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200006
ED Entered STN: 20000706
Last Updated on STN: 20000706
Entered Medline: 20000622
AB It is shown that **calcium** increases the in vitro transfection potency of **plasmid DNA-cationic liposome** complexes from 3- to 20-fold. The effect is **Ca**(2+) specific as other cations, such as Mg(2+) and Na(+), do not give rise to enhanced transfection and the effect can be inhibited by the presence of EGTA. It is shown that **Ca**(2+) increases cellular uptake of the **DNA**-lipid complexes, indicating that increased transfection potency arises from increased intracellular delivery of both **cationic lipid** and **plasmid DNA** in the presence of **Ca**(2+). In particular, it is shown that the levels of intact intracellular **plasmid DNA** are significantly enhanced when **Ca**(2+) is present. The generality of the **Ca**(2+) effect for enhancing complex-mediated transfection is demonstrated for a number of different cell lines and different **cationic lipid** formulations. It is concluded that addition of **Ca**(2+) represents a simple and useful protocol for enhancing in vitro transfection properties of **plasmid DNA-cationic lipid** complexes.

L8 ANSWER 35 OF 66 EMBASE COPYRIGHT 2003 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
AN 2001028876 EMBASE
TI Transfection of BHK cell by serum-stabilized **cationic liposome-DNA** particles.
AU Huang Y.-Y.; Cullis P.R.
CS Y.-Y. Huang, Institute of Biomedical Engineering, National Taiwan University, Taipei, Taiwan,Province of China. yyhuang@ha.mc.ntu.edu.tw
SO Biomedical Engineering - Applications, Basis and Communications, (25 Dec 2000) 12/6 (281-287).
Refs: 10
ISSN: 1016-2356 CODEN: YIGOEO
CY Taiwan,Province of China
DT Journal; Article
FS 030 Pharmacology
037 Drug Literature Index
039 Pharmacy
LA English
SL English
AB Cationic liposomes complexed with **DNA** have been used extensively as non-viral vectors for the intracellular delivery of reporter or therapeutic genes in cell culture and in vivo transfection experiments. Most of **cationic liposome-DNA** particles will be cleared from the blood very quickly when they were administered into the blood circulation system. Serum-stabilized **cationic liposome-DNA** particles made by preformed vesicles and ethanol method were developed [1]. Steric stabilization confers long circulation times to these particles, allowing them to extravasate more easily at sites of porous vasculature. In vitro transfection potency was evaluated by culturing with BHK cells. Experimental results show that the cell uptake of **cationic liposome-DNA** particles made by DODAP/DSPC/Chol /PEG-CerC(14) (25/20/45/10 mol%) was higher than that of made by DODAP/DOPE/Chol /PEG-CerC(14) (20/50/20/10 mol%). However, the transfection efficiency of liposome-**DNA** particles made by DODAP/DOPE/Chol /PEG-CerC(14) (20/50/20/10 mol%) was much higher than the liposome-**DNA** particles made by DODAP/DSPC/Chol /PEG-CerC(14) (25/20/45/10 mol%). This confirms that DOPE is a transfection helper lipid. Except the DOPE, the concentration of **calcium** ion also plays an important role in the BHK transfection experiments. 10 mM **Ca**(++) was necessary for achieving high transfection efficiency.

L8 ANSWER 4 OF 66 MEDLINE on STN DUPLICATE 1
AN 2003144518 MEDLINE
DN 22546492 PubMed ID: 12659962
TI Transfection properties of stabilized **plasmid**-lipid particles containing cationic PEG lipids.
AU Palmer Lorne R; Chen Tao; Lam Angela M I; Fenske David B; Wong Kim F; MacLachlan Ian; Cullis Pieter R
CS Department of Biochemistry and Molecular Biology, University of British Columbia, 2146 Health Sciences Mall, Vancouver, BC, Canada V6T 1Z3.
SO BIOCHIMICA ET BIOPHYSICA ACTA, (2003 Apr 1) 1611 (1-2) 204-16. Journal code: 0217513. ISSN: 0006-3002.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200308
ED Entered STN: 20030328
Last Updated on STN: 20030830
Entered Medline: 20030829
AB Recent work has shown that **plasmid DNA** can be efficiently encapsulated in well-defined "stabilized **plasmid** -lipid particles" (SPLP) that have potential as systemic gene therapy vehicles [Gene Ther. 6 (1999) 271]. In this work, we examine the influence of ligands that enhance cellular uptake on the transfection potency of SPLP. The ligand employed is a cationic poly(ethylene glycol) (PEG) lipid (CPL) consisting of a lipid anchor and a PEG(3400) spacer chain with four positive charges at the end of the PEG (CPL(4)). It is shown that up to 4 mol% CPL(4) can be inserted into preformed SPLP, resulting in up to 50-fold enhancements in uptake into baby hamster kidney (BHK) cells. The addition of **Ca**(2+) to SPLP-CPL(4) (CPL(4)-incorporated SPLP) results in up to 10(6)-fold enhancements in transgene expression, as compared to SPLP in the absence of either CPL(4) or **Ca**(2+). These transfection levels are comparable to those observed for **plasmid DNA-cationic lipid** complexes (lipoplexes) but without the cytotoxic effects noted for lipoplex systems. It is concluded that in the presence of **Ca**(2+) and appropriate ligands to stimulate uptake, SPLP are highly potent transfection agents.

L8 ANSWER 57 OF 66 EMBASE COPYRIGHT 2003 ELSEVIER INC. ALL RIGHTS RESERVED.
on STN DUPLICATE 20
AN 95305375 EMBASE
DN 1995305375
TI Formation of novel hydrophobic complexes between cationic lipids and **plasmid DNA**.
AU Reimer D.L.; Zhang Y.; Kong S.; Wheeler J.J.; Graham R.W.; Bally M.B.
CS Division of Medical Oncology, British Columbia Cancer Agency, 600 West 10th Avenue,Vancouver, BC V5Z 4E6, United States
SO Biochemistry, (1995) 34/39 (12877-12883).
ISSN: 0006-2960 CODEN: BICHAW
CY United States
DT Journal; Article
FS 027 Biophysics, Bioengineering and Medical Instrumentation
029 Clinical Biochemistry
037 Drug Literature Index
LA English
SL English
AB An ability to generate a well defined lipid-based carrier system for the delivery of **plasmid DNA** in vivo requires the characterization of factors governing **DNA**/lipid interactions and carrier formation. We report that a hydrophobic **DNA**/lipid complex can be formed following addition of cationic lipids to **DNA** in a Bligh and Dyer monophase consisting of chloroform/methanol/water (1:2.1:1). Subsequent partitioning of the monophase into a two-phase system allows for the extraction of **DNA** into the organic phase. When using monovalent cationic lipids, such as dimethyldioctadecylammonium bromide, dioleyldimethylammonium chloride, and 1,2-dioleyl-3-N,N,N-trimethylaminopropane chloride, greater than 95% of the **DNA** present can be recovered in the organic phase when the lipid is added at concentrations sufficient to neutralize **DNA** phosphate charge. When the polyvalent cationic lipids 2,3-dioleyloxy-N-[2(sperminecarboxamido)ethyl]- N,N-dimethyl-1-propanaminium trifluoroacetate and diheptadecylamidoglycyl spermidine are used, efficient extraction of the **DNA** into the organic phase is also achieved when the charge ratio between lipid and **DNA** is approximately equal. Formation of the hydrophobic **DNA** complex can only be achieved with cationic lipids. In the absence of added cations or in the presence of excess Ca2+, L-lysine, or poly(L-lysine), 100% of the **DNA** is recovered in the aqueous fraction. The monovalent **cationic lipid/DNA** complexes can also be prepared in the presence of detergent; however, low concentrations of NaCl (<1 mM) lead to dissociation of the complex. Importantly, these results clearly demonstrate that **cationic lipid** binding does not lead to **DNA** condensation. The methods described, therefore, enable **DNA**/lipid complexes to be characterized in the absence of **DNA** condensation. It is believed that this approach, where cationic lipids added in monomeric or micellar form are bound to **DNA** prior to condensation, will facilitate the preparation of **DNA**/lipid complexes with well defined surface characteristics and size.

L6 ANSWER 34 OF 56 MEDLINE on STN DUPLICATE 12
AN 1999227116 MEDLINE
DN 99227116 PubMed ID: 10209255
TI **Calcium** *ions as efficient cofactor of polycation-mediated gene transfer.*
AU Haberland A; Knaus T; Zaitsev S V; Stahn R; Mistry A R; Coutelle C; Haller H; Bottger M
CS Franz Volhard Clinic at the Max Delbruck Center for Molecular Medicine, Wiltberg Strasse 50, D-13122, Berlin-Buch, Germany.
SO BIOCHIMICA ET BIOPHYSICA ACTA, (1999 Apr 14) 1445 (1) 21-30.
Journal code: 0217513. ISSN: 0006-3002.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199905
ED Entered STN: 19990607
Last Updated on STN: 20030118
Entered Medline: 19990527
AB We investigated the effect of **calcium** on the transfection of non-viral DNA transfer systems. Cationic proteins such as the nuclear protein H1, the polycation polylysine and a number of commercial transfection agents exhibited high transfection rates in the presence of Ca2+. Without Ca2+ H1 and HMG1 were inactive in transfection of the human permanent endothelial cell line ECV 304 while cationic liposomes such as **Lipofectin** and Lipofectamine did not show any Ca2+ dependence. More detailed experiments showed that Ca2+ was replaceable by the lysosomotropic agent chloroquine. Furthermore, it was possible to separate the transfection-enhancing role of Ca2+ from the actual transfection process by adding Ca2+ to the cells after the transfection period and still to obtain a significant transgene expression. This makes it possible to distinguish between cellular uptake of H1 (or mediator)-DNA complexes and endocytotic release. We also replaced soluble Ca2+ by **Ca**-phosphate precipitates not containing DNA and obtained similar transfection results. This allowed us to suggest that the addition of free Ca2+ to the transfection medium resulted in nascent **Ca** -phosphate microprecipitates. The known fusogenic and membranolytic activity of such microprecipitates could facilitate the transport through and the release of the transfecting complexes from the **endosomal** /lysosomal compartment.

L8 ANSWER 49 OF 66 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI on STN
AN 1998-00956 BIOTECHDS
TI Transfection of human endothelial cells;
reporter gene transfer to human umbilical vein endothelial cell using biolistic particle bombardment, **cationic liposome**, **calcium** phosphate and DEAE-dextran for cardiovascular disease gene therapy
AU Tanner F C; Carr D P; Nabel G J; *Nabel E G
CS Univ.Michigan; Howard-Hughes-Med.Inst.
LO Department of Internal Medicine, University of Michigan, 1150 W. Medical Center Drive, 7220 MSRB III Ann Arbor, MI 48109-0644, USA.
SO Cardiovasc.Res.; (1997) 35, 3, 522-28
CODEN: CVREAU ISSN: 0008-6363
DT Journal
LA English
AB Human umbilical vein endothelial cell transfection was investigated. *Transfections by particle-mediated gene transfer* (biolistic particle bombardment) or by cationic liposomes were optimized and compared to **calcium** phosphate and DEAE-dextran. Transfection efficiency was determined using a beta-galactosidase (EC-3.2.1.23) or placental alkaline phosphatase (EC-3.1.3.1) reporter gene. The effect of promoter strength was analyzed by transfecting plasmids with either the Rous-sarcoma virus or cytomegalo virus promoter regions. Optimal conditions for particle-mediated gene transfer utilized gold particles of 1.6 um diameter, a target distance of 3 cm, helium pressure of 8.96 MPa (1,300 psi) and cell confluence of 75%. Transfection with different cationic liposomes demonstrated that gamma-AP-DLRIE/DOPE was optimal for gene transfer when 5 ug of **DNA** and 10-20 ug of lipid was used. With both gold particles and the liposome, alkaline phosphatase was more efficient than beta-galactosidase. Optimum gene transfer efficiency was 20.28% of cells with the liposome, 3.96% with biolistics, 2.09% with **calcium** phosphate and 0.88% with DEAE-dextran. (19 ref)